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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

On 10 March 2004

TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Dajic



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Wilhelm Gruissem

Application No.: 09/134,014

Filed: August 14, 1998

For: HOMOLOGOUS  
RECOMBINATION IN PLANTS

Customer No.: 20350

Confirmation No. 7338

Examiner: David T. Fox

Technology Center/Art Unit: 1638

DECLARATION OF DR. JOHN G.  
JELESKO. §1.132

Commissioner for Patents  
Alexandria, VA 22313-1450

Sir:

I, Dr. John G. Jelesko, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. I received a Ph.D. in 1992 from the University of Washington.
2. I am currently employed as an Assistant Professor in the Department of Plant Pathology, Physiology and Weed Science at the Virginia Polytechnic Institute and State University,

Blacksburg, Virginia. I have worked in the field of plant molecular biology for over 10 years. A copy of my curriculum vitae is attached as Exhibit A.

3. I have read and am familiar with the contents of the application. The claims currently under examination are drawn to a method of identifying homologous recombination in plant cells. As I understand it, the claims have been rejected as allegedly too broad. The Examiner alleges that it would require undue experimentation to develop and evaluate methods of identifying homologous recombination in plants cells with a genetic construct that does not contain a selectable marker gene. In particular, the Examiner contends that the claims are only enabled for a method that involves an additional step of identifying transformed plant cells by culture on a selective medium prior to the step of detecting the presence of reporter activity in the plant cells. In this Declaration, I will present additional data that a selection step is not required prior to screening for reporter activity. The work was performed by me or under my supervision.

4. We generated a synthetic RBCSB gene cluster (*synthRBCSB3*) to investigate somatic DNA recombination events. This construct was designed such that the conditional expression of a firefly luciferase gene was dependent on a cross-over event between the paralogous *RBCSB* gene. To generate large populations of *Arabidopsis* seedlings containing the *synthRBCSB3* gene cluster, Col-0 plants were transformed using a floral vacuum infiltration method using GV3101 containing pJGJ205. To maximize the likelihood of obtaining luc+ seedlings derived from independent recombination events, the plant transformation and seed collection protocols were organized to maximize independent seed lots. Each pot of T0 Col-0 plants was infiltrated once every seven days for three weeks and then placed in trays until all inflorescences turned brown.. Each infiltration experiment consisted of 3 trays containing 17 pots with 9 plants per pot (approximately 460 T0 plants per infiltration). To insure the isolation of independent T1 seedlings, T1 seeds were collected from each tray into three independent lots, resulting in at least nine separate T1 seed lots.

5. To determine the approximate number of transformed seedlings in the T1 generation, a representative sample (110 mg) of each T1 seed lot was surface sterilized with 50% bleach and plated on 0.5 X MS media containing 1X Gamborg's B5 Vitamins and 50 µg/ml Kanamycin Sulfate (AgriBio, North Miami, Florida); the observed number of Kmr seedlings was used to determine the frequency of Kmr seedlings per mg seed in each lot. These values were used to estimate the total number of Kmr seedlings observed in the luciferase screen, and provides a basis for estimating the transformation frequency for the experiment described here. This does not constitute a selection step performed prior to screening for activity. The frequency of T-DNA transformation varied from lot to lot and ranged from  $0.7-8.9 \times 10^{-3}$ , which is similar to that obtained by others.

6. Luciferase positive seedlings were identified as follows. Approximately, 150-200 mg of seed (approximately 7,500 to 10,000 seed) were evenly distributed on a 20 cm x 20 cm piece of Whatman 3MM chromatography paper resting on felt pads saturated with 50 ml of 1X Hoaglands Solution in a Jiffy half tray with dome. Trays were incubated for two nights at 4° C and then incubated under fluorescent lights on a 16 hour light 8 hour dark cycle for five to seven days. Each tray was assayed for *in vivo* luciferase activity and luciferase positive seedlings were isolated. Each screening session included one tray containing about 10-20 transgenic AtJGJ204.7 seedlings, containing the full length *RBCS1B* promoter *RBCS1B::LUC* gene fusion, to serve as a positive control for the luciferase imaging equipment. Genomic DNA from luciferase positive plants was isolated by the CTAB method for plant genomic DNA to characterize chimeric genes present in the luciferase-positive seedlings. The screen of the plants infiltrated with the plasmid comprising the using *synthRBCSB3* gene yielded sixteen luciferase-positive seedlings identified from 5.9 million seedlings. Of these, nine were formed by homologous recombination between paralogous *RBCSB* sequences associated with T DNA integration.

7. In summary, the experiments described here provide additional data that a selectable marker is not required in order to identify plants that exhibit reporter activity in which homologous recombination has occurred.

8. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

Dated: \_\_\_\_\_  
John Jelesko, Ph.D.

**John G. Jelesko**

Plant Pathology, Physiology, and Weed Science  
Virginia Polytechnic Institute and State University  
Blacksburg, VA 24061-0331  
voice: 540/231-3728  
fax: 540/231-7477

**PERSONAL DATA:**

Citizenship: United States of America

**EDUCATION:**

1992 Ph.D. University of Washington.  
1988 M.S. University of Washington.  
1983 B.S. University of California, Davis.

**RESEARCH EXPERIENCE:**

2000- Assistant Professor, Virginia Polytechnic Institute and State University, Blacksburg, VA  
1998-00 Associate Specialist, Dr. Wilhelm Gruissem, University of California, Berkeley.  
1997-98 Visiting Researcher, Dr. Masaki Furuya, Hitachi Advanced Research Labs Ltd., Japan.  
1992-98 Postdoctoral Fellow, Dr. Wilhelm Gruissem, University of California, Berkeley.  
1986-92 Graduate Student, Dr. John Leigh, University of Washington, Seattle, WA.  
1983-86 Chemist II, Dr. Thomas Kempe, SYVA Co., Palo Alto, CA.  
1982-83 Undergraduate research project, Dr. Sydney Kustu, University of California, Davis, CA.

**FELLOWSHIPS and GRANTS:**

2000-01 NSF Small Grant for Exploratory Research  
2001-05 NIH General Medical Sciences Grant  
2000-02 National Science Foundation US Japan Cooperative Science Grant  
2000-02 Virginia Grant for Medicinal Tobacco Research  
1996-97 National Science Foundation / Center for Global Partnership supplemental travel award.  
1994-97 National Science Foundation Postdoctoral Fellowship in Plant Biology.  
1991-92 U.W. Predoctoral Plant Molecular Integration and Function Training Grant, competitive position.  
1987-91 U.S. Public Health Service Predoctoral Traineeship in Developmental Biology, competitive position.

**PUBLICATIONS :**

**Jelesko, J. G.,** K. Carter, W. Thompson, Y. Kinoshita, W. Gruissem. Meiotic recombination between paralogous *RBCSB* genes on sister chromatids of *Arabidopsis thaliana*. *Genetics*. (in press)  
**Deng, F., J. Jelesko, C. L. Cramer, J. Wu, K. K. Hatzios.** 2003. Use of an antisense gene to characterize glutathione S-transferase functions in transformed suspension-cultured rice cells and calli. *Pesticide Biochemistry and Physiology*. 75:27-37.  
**Jelesko, J.,** R. Harper, M. Furuya, and W. Gruissem. 1999. Unequal crossing-over leading to gene duplication and chimeric gene formation in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA*, 96:10302-10307.  
**Jelesko, J.,** S. M. Jenkins, M. Rodríguez-Concepción, and W. Gruissem. 1998. Tomato *HMGI* is expressed in tissues undergoing cell division and growth. *Planta*, 17:73-82.  
**Jelesko, J. and J. Leigh.** 1994. Genetic characterization of a *Rhizobium meliloti* lactose utilization locus. *Mol. Microbiol.* 11:165-173.  
**Jelesko, J., J. Lara, and J. Leigh.** 1993 *Rhizobium meliloti* mutants with Decreased DAHP synthase activity are sensitive to exogenous tryptophan and phenylalanine and form ineffective nodules, *Mol Plant Microbe Interactions*, 6:135-143.

- Ullman, E. F., G. Milburn, **J. Jelesko**, K. Radika, M. Pirio, T. Kempe, and C. Skold. 1993. Anti-immune complex antibodies enhance affinity and specificity of primary antibodies. *Proc. Natl. Acad. Sci. USA*, 90:1184-1189.
- Kustu, S. K., J. Hirshman, D. Burton, **J. Jelesko**, and J. Meeks. 1984. Covalent modification of bacterial glutamine synthetase: physiological significance. *Mol. Gen. Genet.* 197:309-317.

#### ABSTRACTS:

- J. Jelesko**, Y. Kinoshita, W. Thompson, M. Furuya, and W. Gruissem. 2002. Frequency and position of meiotic and mitotic unequal crossing-over events within a synthetic RBCSB gene cluster. XIII International Conference on Arabidopsis Research, Sevilla, Spain.
- F. Deng, **J. Jelesko**, C. Cramer, Jingrui Wu, and K.K. Hatzios, 2002. Use of antisense gene to characterize the physiological functions of glutathione S-transferases in transgenic rice seedlings and Arabidopsis seedlings. Annual Meeting of Weed Science Society of American. Reno, Nevada (February 10-14, 2002).
- F. Deng, **J. Jelesko**, C. Cramer, and K.K. Hatzios, 2002. Use of antisense gene to characterize the physiological functions of glutathione S-transferases in transgenic rice seedlings and Arabidopsis seedlings. Annual Meeting of Southern Weed Science Society. Atlanta, Georgia. (January 28-30, 2002).
- Reed, D., N. Kuno, K. Uchida, M. Furuya, and **J. Jelesko**. 2001 Alkaloid Biosynthetic Gene Discovery Using High Throughput Fluorescent Differential Display. Phytochemical Society of North America Annual Meeting. (August 4-8, 2001)
- J. Jelesko**, R. Harper, M. Furuya, and W. Gruissem. 1998. Detection of rare meiotic unequal cross-over events leading to gene duplication and recombinant genes. Proceedings from The Arabidopsis Genome: a Model for Crop Species, Cold Spring Harbor Laboratory. New York.
- J. Jelesko**, R. Harper, M. Furuya, and W. Gruissem. 1998. Detection of rare meiotic unequal cross-over events leading to gene duplication and recombinant alleles. 1998proceedings from the International Conference on Arabidopsis Research, Madison, Wisconsin.
- J. Jelesko**, R. Harper, M. Furuya, and W. Gruissem. 1998. Unequal crossing over leading to gene duplication and chimeric gene formation in *Arabidopsis thaliana*. Proceedings from the Gordon Research Conference on Molecular Evolution, Ventura, California.
- J. Jelesko**, S. M. Jenkins, and W. Gruissem. 1996. HMG1 is induced during cell division and growth. Proceedings from the Molecular Biology of Tomato. Berkeley California
- J. Jelesko** and J. Leigh. 1992. *Rhizobium meliloti* locus affecting growth in the presence of aromatic amino acids and bacteroid development. Proceedings from the Sixth International Symposium on Molecular Plant-Microbe Interactions.
- Milurn, G. L., **J. Jelesko**, M. Pirio, and T. Kempe. 1989. A monoclonal anti-idiotypic complex antibody specific for the ligand binding site of another monoclonal antibody. *F.A.S.E.B.*, 3:A1110.
- J. Jelesko** and J. Leigh. 1988. Genetic analysis of lactose utilization and succinate catabolite repression in *Rhizobium meliloti*. Proceedings from the Northwest Regional American Society for Microbiology meeting. Seattle, Washington
- Bartlett, C., **J. Jelesko**, S. Pankey, A. Izutsu, T. Kempe, C. Collins, and A. Jakalich. 1986 Desipramine monoclonal antibody for quantization in serum. *Clin. Chem.* 32:1064.

#### TEACHING EXPERIENCE:

- 2003 PPWS 2984 (special studies) *Domesticating the Gene*, 3 credit hours, team taught 75% instruction effort.
- 2002 PPWS 5004 *Graduate Seminar*, 1 credit hour. 100% instruction effort
- 2002 PPWS 2984 (special studies) *Domesticating the Gene*, 3 credit hours, team taught 60% instruction effort. applied for Regular and University Core Curriculum course approval.
- 2001 PPWS 2984 (special studies) *Domesticating the Gene*, 3 credit hours, team taught 60% instruction effort. New course.
- 2000 ALS/PPWS 6024, *Topics in Molecular Cell Biology and Biotechnology*, 3 credit hours, 13% instruction effort.
- 1989 MICRO 431. Univ. of Washington. Guest Lecturer. 7% Instructional effort
- 1987 BIO101, Univ. of Washington. Teaching Assistant. 100% Instructional effort.

1986 MICRO 401. Univ. of Washington. Teaching Assistant. 100% Instructional effort..

#### **PRESENTATIONS**

- 2002 Third Annual Arabidopsis Mini-Symposium, Univ. Maryland College Park, "*Investigating Meiotic Recombination one photon at a time*". April 13<sup>th</sup>
- 2001 Virginia Tech Biochemistry Department Seminar, *New methods to investigate meiotic recombination at complex loci*, November 12<sup>th</sup>.
- 2001 Phytochemical Society of North America Annual Meeting., Oklahoma City, "*Alkaloid Gene Discovery Using High Throughput Differential Display*" August 4-8<sup>th</sup> 2001
- 2001 Virginia Tech Botany Seminar speaker: *Investigating recombination one photon at a time*. Feb 9<sup>th</sup>.
- 2000 Virginia Tech Interdepartmental Plant Physiology Program seminar: *Meiotic recombination within complex loci*. February 24<sup>th</sup>.
- 1997 Research Institute for Biological Sciences, Okayama, Japan. (Lecture) Identification and isolation of rare homologous recombination events in *Arabidopsis thaliana*. October 14th

#### **PATENTS:**

- 1993 United States Patent No 5,223,441. Receptors for Immune Complexes, E. Ullman, **J. Jelesko**, M. Pirio, D. Gould, and T. Kempe.

#### **PROFESSIONAL SOCIETIES:**

International Society of Plant Molecular Biologists  
Phytochemical Society of North America  
American Society of Plant Biologists  
American Association for the Advancement of Science  
Phi Beta Delta, Honor Society for International Scholars